Reproductive assurance through unusual autogamy in the absence of pollinators in *Passiflora edulis* (passion fruit)

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*Passiflora edulis* (passion fruit), a native of Brazil, is widely grown in frost-free areas around the world. In its native habitat and many other areas, the species is self-incompatible and carpenter bees are the effective pollinators. In India, it is cultivated in North East and southern states. In two of the populations of *P. edulis*, *F. flavicarpa* (yellow passion fruit) in Karnataka, there was good fruit set, although there were no effective pollinators. Studies were carried out on reproductive ecology of yellow passion fruit to understand the mode of fruit set in the absence of pollinators and the breeding system. Although several floral visitors, *Apis cerana*, *Trigona* sp. and an ant (*Camponotus* sp.) visited the flowers, they were all nectar robbers and not the pollinators. There is a unique autonomous self-pollination, involving the movement of anthers and stigmas, during flower opening which provides reproductive assurance to the species even in the absence of pollinators. The populations used in the present study are self-compatible. Thus, the species is able to produce constant fruit set even when the population size is small and pollinators are absent; evolution of these features seems to be the main factor for its wide distribution around the world.

**Keywords:** Autogamy, *Passiflora edulis*, passion fruit, reproductive assurance.

*Passiflora* (Passifloraceae) is a large genus comprising over 400 species. *Passiflora edulis* Sims (passion fruit), native to Brazil, is widely cultivated in frost-free areas in over 20 countries around the world, including India, Sri Lanka and Bangladesh, for its edible fruits and ornamental flowers. The plant is a perennial woody climber and its fruits are attractive for their decorative color, flavor and tasty juice. In India it is cultivated in the North Eastern and southern states. The fruits form the main economic product and are used in the preparation of juice, which is rich in vitamins, minerals and carotene. The juice is consumed alone or mixed with other juices to improve the aroma. In several countries, the juice is also used to flavor many other food products such as desserts, jams, jellies, ice creams, pastries and yogurt. The species also has considerable medicinal properties. In traditional system of medicine its fruits are used as sedative, antiasthmatic and emetic, and its leaves are used in the treatment of insomnia, epilepsy, ulcers and hemorrhoids. The leaves contain a bitter principle, maracugine, and resins, acids and tannins. The fruits show strong antioxidant and bactericidal properties.

Passion fruit is perhaps one of the most widely grown fruit crops around the world. Since the extent of fruit set is dependent on effective pollination, the species is expected to have an efficient pollination mode and flexible breeding system for such a wide adaptability. Apart from bees, humming birds and bats have been reported to be the pollinators of different species of *Passiflora*. In its native Brazil and West Indies, *P. edulis* is self-incompatible and *Xylocopa* species are the effective pollinators. In Australia, commercially managed honey bees are used for pollination services. In North East India, *Apis mellifera*, *A. cerana* and *Xylocopa* spp. have been reported to be the most common floral visitors. *P. edulis* has two varieties – *P. edulis* *F. edulis* (purple passion fruit) and *P. edulis* *F. flavicarpa* (yellow passion fruit); the former is generally grown at higher elevations and the latter at lower elevations.

During my preliminary observations on *P. edulis* *F. flavicarpa* in the beginning of the flowering season (May–June) of 2011, I did not find any effective pollinator, but the plants produced good number of fruits. To find answers to the mode of fruit set in the absence of effective pollinators, I studied reproductive ecology of *P. edulis* *F. flavicarpa* with particular focus on the mode of natural pollination, if any, its efficacy and the breeding system.

Studies were carried out during the reproductive phase of 2011 and 2012 on plants cultivated at Odekar Farm (population-1) located 30 km from Tumkur, Karnataka (13°31′11″N, 72°2′39″E). During 2012, the studies were extended to another population (population-2) located at Pura (13°9′35″N, 76°53′39″E), near Nittrur, also in Tumkur district, about 45 km away from population-1. Flowering phenology was studied through daily observations from the time the flower buds became visible. To study details of anthesis, anther dehiscence and longevity of the flower, 30 flower buds (10 each in three replicates) that would open the next day were tagged and kept under hourly observations from 06.00 h until the flowers closed. As initial studies revealed that flowers open around 12.30–13.00 h and the process is very rapid completing in <15 sec, tagged flower buds were kept under continuous observation from 12.00 h until the completion of anthesis. During anthesis, the movement of the stamens and stigmas and their position with reference to each other were monitored carefully. The amount of nectar secreted was measured in bagged flowers at 18.00 h using microcapillaries (25 μl, Drummond). Sugar equivalent of the nectar was determined using a portable refractometer. To estimate the number of pollen grains per anther, each anther (*N* = 10), before dehiscence, was carefully scraped under...
Figure 1. Floral morphology, floral visitors and autogamous pollination in *Passiflora edulis* F. flavicarpa. *a*, Flower photographed from the top. *b*, *Apis cerana* foraging the nectar. *c*, *Camponotus* sp. foraging the nectar. Anthers have been removed before photography. *d* and *e*, Flowers photographed soon after anthesis to show autogamous pollination of one stigma (*d*) and of two stigmas (*e*). Regions where anthers touch the stigma are shown by arrows. *f*, Ripened fruits.

a stereomicroscope into 2 ml of water containing a few drops of a detergent (to prevent clumping of pollen grains) taken in a well of a titre plate to release all the pollen grains; the debris was removed under a stereomicroscope. Then 0.2 ml of pollen suspension (*N* = 3 for each anther) was spread on a slide in the form of a narrow band and allowed to dry. A graph paper was stuck below the slide and the number of pollen grains was counted along the grids of the graph paper under a binocular stereomicroscope fitted with an incident light source. The scores were computed to calculate the number of pollen grains per anther. To count the number of ovules in each ovary (*N* = 10), the ovary was cut longitudinally into four parts and the ovules from each part were scraped into 2 ml water containing a few drops of a detergent. The suspension was spread on four slides (0.5 ml/slide) and the number of ovules was counted after sticking a piece of graph paper below the slide.

Floral visitors were observed from the time of flower opening until its closure in 15 min time slots each hour. Details of their approach, feeding pattern and their movement in relation to the position of the anthers and the stigma were carefully monitored. The breeding system was studied through manual self- (pollen from the same flower/vine) and cross- (pollen from another vine) pollinations (*N* = 20 each). Pollinations were carried out on all the three stigmas, bagged and monitored for fruit set. The significance between the pollination treatments was analysed using two-tailed independent *t*-test with equal variance assumed at 0.05 probability level. All data were analysed using SPSS 16 statistical package.

The flowering was initiated by the end of June or early July; the vines continued to flower until the end of July or first week of August. The flowers open between 12.30 and 13.30 h. The anthers dehisce before flower opening. The flowers are large (mean length 2.75 ± 0.10 cm and mean width 7.28 ± 0.17 cm; Figure 1 *a*), solitary and borne in the axils of leaves. The flowers are conspicuous mainly because of the purplish corona. The flower has a 3–4 cm long pedicel bearing three green, leafy bractioles at the tip. Sepals are five, free, green on the outer surface and white on the inner surface. The outer surface of the sepals bears one or two dark, extra floral nectarines at the margins. There are five free, white linear petals. Inside the petals are two whorls of densely arranged corona hairs, the proximal part of which is purplish and the distal part white. The sepals, petals and the corona are fused at the base to form about 1 cm deep cup surrounding the swollen basal part of the gynandrophore. The gynandrophore (1.21 ± 0.07 cm long) is surrounded by the nectary in the
form of a ring at the base. The gynandrophore bears five stamens and the pistil at the tip. The ovary (about 1 cm long) produces three robust styles (mean length 1.78 ± 0.07 cm), each terminating in a swollen, slightly bi-lobed stigma. In the bud stage, the styles are erect and form a central column. Anthers are versatile, introrse and are adpressed to the stylar column in the bud. In opened flowers, the stamens spread out and the anthers turn around to bring the dehisced surface facing down. The styles also spread out and position horizontally; the receptive surface of the stigma faces down (Figure 1a). The stigmatic surface is green, dry and covered with densely arranged papillae. Each anther produces 25,070.33 ± 827.54 pollen grains. The number of pollen grains produced per flower works out to be 125,350. Pollen grains are comparatively large and measure 57.69 ± 3.87 μm in diameter.

There was no visible nectar in freshly opened flowers. The flowers started secreting nectar after anthesis and by 3.00 p.m. the nectar was clearly visible in bagged flowers. It steadily increased and by 6.00 p.m. it reached 99.5 ± 19.5 μl per flower (N = 10). The concentration of sucrose in the nectar was 18.95 ± 1.09%. The distance between the corona and the exposed lower surface of the anthers was 10 ± 0.89 mm and that between the corona and the receptive surface of the stigma was 13.83 ± 1.16 mm. The ovary is tricarpellary with a single locule. The number of ovules per ovary (N = 10) was 300 ± 13.95, borne on three parietal placentae. The pollen to ovule ratio works out to be 417.8 : 1. Pollen grains and nectar are exposed, and are readily accessible to any floral visitor.

Honey bee, *Apis cerana* was the only floral visitor to the flowers of population-1 throughout the flowering period of 2011. They started visiting the flowers soon after anthesis and continued until the evening (18.00–18.30 h). The bees landed on the flat corona and foraged nectar (Figure 1b) located at the base of the gynandrophore; they never foraged pollen. As the bees kept consuming nectar as it was secreted, the nectar was hardly visible in the unbagged flowers. Often, 2–4 bees could be seen on the same flower simultaneously foraging the nectar. Occasionally, the upper part of the approaching bee came in contact with the lower exposed pollen-bearing surface of the anther and some pollen grains got deposited on the wings and thorax of the bee. However, the bee never touched the stigma, which is located at a much higher level.

During the 2012 flowering season, however, *A. cerena* never visited the flowers during the initial and mid phase of flowering. One ant species, *Camponotus* sp. (Formicinae), however, was present throughout the flowering period. Ants were present at the bud stage itself and foraged from the dark, extra-floral nectaries located on the sepalas. As the flower opened, they started foraging the nectar from the flowers. Each flower was generally foraged by 2–3 ants (Figure 1c). Ants visited most of the flowers from the time of opening until they closed. Although the ants moved around the flower, they never touched the exposed part of the anther or the stigma. *A. cerana*, which was absent until the last phase of flowering, started visiting the flowers only during the last week of flowering and foraged nectar. The bees avoided the flowers that were being foraged by ants. Often they approached such flowers but flew away without landing or immediately after landing without foraging the nectar.

In population-2, ants as well as *A. cerana* were absent. However, a stingless bee, *Trigona* sp. visited the flowers in high frequency and foraged predominantly the nectar (about 80% of the visits) and occasionally pollen. Often 2–3 individuals visited each flower simultaneously. As in population-1, none of the bees came in contact with the stigma and did not bring about pollination. These studies highlight temporal and spatial variation in floral visitors, which is well-recognized in the literature on pollination ecology. Although the morphology of the flower is suited for pollination by larger bees, particularly the carpenter bee (*Xylocopa* spp.), they never visited the flowers of *P. edulis* in both the populations. Thus, the present study clearly showed that there were no effective pollinators in both the populations studied. *A. cerana* and ant species were nectar robbers, and *Trigona* sp. robbed both the nectar and the pollen. This was confirmed by the absence of seed set in any of the emasculated (but unbagged) flowers (Table 1).

### Table 1. Fruit set in naturally pollinated (autogamous) and manually pollinated flowers in *Pas-siflora edulis* *F. flavicarpa*. Significance of each manual pollination treatment (one, two and three stigmas) was analysed independently with autogamous pollination using *t*-test

<table>
<thead>
<tr>
<th>Pollination treatment</th>
<th>Fruits set (%)</th>
<th><em>t</em>-value (<em>P</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural autogamous self-pollination</td>
<td>60.0</td>
<td></td>
</tr>
<tr>
<td>Manual pollination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One stigma pollinated</td>
<td>87.5*</td>
<td>−2.18 (0.03)</td>
</tr>
<tr>
<td>Two stigmas pollinated</td>
<td>92.8*</td>
<td>−2.82 (0.007)</td>
</tr>
<tr>
<td>Three stigmas pollinated</td>
<td>83.4*</td>
<td>−3.18 (0.002)</td>
</tr>
<tr>
<td>Emasculated and bagged (for apomixes)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Emasculated and unbagged (for insect pollination)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Significant over natural autogamous pollination.
There is no possibility of autogamy in opened flowers as the stigma and anthers do not touch each other (Figure 1a). The possibility of apomixis (seed development without fertilization) was ruled out as none of the emasculated and bagged flowers (N = 20) developed into fruits; all of them abscised within 3–4 days. To find out if there is any possibility of autogamy at the bud stage, careful observations were made on the position of the anthers and the stigma. During anthesis, the sepals, petals, stamens and styles spread out rapidly. The receptive surface of the stigma moves down and the anther filaments spread out. During this process each anther tumbles down at the tip of the filament and turns around, thus bringing the dehisced side of the anther facing down (Figure 1d). During this movement of the anthers and styles, a part of the pollen-bearing surface of one (Figure 1e) or two anthers (Figure 1f) comes in contact with the receptive surface of the stigma located just above the respective anthers and deposits some pollen grains onto the stigmatic surface before the anther moves down completely. In none of the flowers could I observe all the three stigmas getting pollinated. As the pollen grains are bright yellow and large, and the stigmas are dark green, the presence of pollen grains on the stigma could be clearly seen with a hand lens. Only one stigma got pollinated in 43.7% (N = 40) of the flowers and two stigmas were pollinated in 25% of the flowers and the remaining flowers remained unpollinated. Thus, autogamous pollination efficiency turned out to be 68.7%. In population-2 also, the mechanism of autogamous pollination was similar to that in population-1. Thus, in both the populations studied, flowers have an intrinsic mechanism to achieve autonomous self-pollination.

The flowers remain open for about 10–12 h and close by midnight. The styles start to become erect by 18.00 h (about 5 h after anthesis) and become erect by 20–22 h. By this time the sepals and petals also lose turgidity and start closing. The petals dry up in about 4 days and the sepals remain green for 7–10 days before they dry up. However, dried sepals and petals remain attached to the stalk of the developing fruits and are present even in ripened fruits. Bracteoles remain fresh and green even in ripened fruits. Abscission zone develops just above the bracteoles and results in the abscission of the ripened fruits. The stigmas and styles dry up in about a week after flower opening, but remain attached to the tip of the fruit even in ripened fruits.

Unpollinated flowers abscise within 3–4 days after anthesis without showing any enlargement of the ovary. Pollinated flowers show rapid enlargement of the ovary and developing fruits can be clearly recognized by three days after anthesis. The fruits turn green after they emerge from the surrounding sepals and petals. To check if there is any pollination limitation under natural conditions, natural fruit set was compared with that in manually pollinated flowers (Table 1). Although over 68% of the flowers got pollinated through autonomous selfing, only 60% fruit set was recorded. This may be due to limited number of pollen being deposited in some of the flowers; as the fruit contains a large number of seeds, fertilization of a minimum number of ovules may be necessary to initiate and sustain fruit development. Manually pollinated flowers yielded over 80% fruit set, irrespective of the number of stigmas pollinated (Table 1). The difference in the extent of fruit set between autogamous and manually pollinated (1, 2 and 3 stigmas) flowers was significant indicating pollination limitation to some extent in natural autogamy.

Developing fruits attained maximum size in three weeks, but took another 5–6 weeks (about 8–9 weeks from the day of flower opening) to ripen. Ripened fruits are oval, bright yellow with glossy surface and show marked variation in their size, weight and seed number. The average size of fruits obtained in natural autogamous pollinations was significantly lower when compared to those obtained in manual pollination (Figure 2). This may be due to lesser number of pollen deposited in some of the autogamously pollinated flowers and consequent...
development of lower number of seeds resulting in smaller fruits in such flowers. There was a positive correlation between the fruit size (length × diameter), fruit weight and seed number (Figure 3), irrespective of pollination treatments. There was no correlation between the number of stigmas pollinated and the size/weight/seed number of fruits. Pollination of one stigma was thus sufficient to yield full fruit and seed set. This is obviously because the pollen tubes coming from any stigma get distributed to ovules of all the carpels as the transmitting tissues of all the styles (through which the pollen tubes grow) are fused just above the ovarian cavity.

There was no difference in fruit and seed set between selfed and crossed flowers, demonstrating that the species is self-compatible. Although *P. edulis* in its native habitat is self-incompatible, both the populations studied have become self-compatible. The change from self-incompatibility to self-compatibility has frequently occurred in flowering plants, particularly in cultivated species. 16,17

Towards the end of the flowering season, some abnormalities were recorded. All the three styles remained erect in opened flowers and did not bend down in about 20% of the flowers. The proportion of flowers in which styles remained erect reached almost 50% in the last few days of flowering. Such flowers did not get pollinated and did not set fruits. About 10% of the developing fruits shrivelled; this seems to be due to resource constraint to supply nutrients to all the developing fruits.

A number of species have evolved various devices to achieve autogamous self-pollination18 when biotic pollination is a limitation. Some of them are mediated through floral visitors19 and others are independent of floral visitors20–22. Autogamous self-pollination has evolved frequently in orchids (Chen et al.23 and references therein), many of which generally grow in pollinator-scarce habitats. Evolution of autogamous self-pollination has generally been interpreted as a means of reproductive assurance in the absence of effective pollinators24. The present study clearly shows that autonomous selfing during flower opening is the only method of pollination in the populations of *P. edulis* studied. This ensures constant level of fruit production even when populations are small and pollinators are absent. It does not compromise cross-pollination; wherever pollinators are available, it permits mixed mating. To my knowledge, such autogamous selfing has not been reported in any other species. Even in *Passiflora*, there are no reports of such autogamous pollination either in *P. edulis* or in any other species. It is not clear whether this feature is intrinsic to *P. edulis* (but has gone unnoticed by early investigators) or has evolved as a result of pollinator scarcity in some of the populations. As the species growing in its native habitat is reported to be self-incompatible (SI)10, autogamous self-pollination, even if it is intrinsic to *P. edulis*, does not result in fruit set in SI populations and does not provide reproductive assurance. Evolution of self-compatibility and autonomous selfing which ensures constant seed set in small populations and under variable pollination environments is probably the main feature contributing to such a wide adaptation of the species around the world.

Analysis of the potential of kimberlite rock spectra as spectral end member using samples from Narayanpet Kimberlite Field, Andhra Pradesh

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Mineral spectra as an end-member have been used for spatial mapping of the mineral deposits and associated potential lithovariants (altered rocks, etc.) while processing the remotely sensed hyperspectral data. But in nature, minerals occur in a mixture in rocks. Therefore, characterization of rock spectra is important. Here we study the role of texture, grain size and relative mineralogical abundances of constituent minerals in shaping the spectral features of the rock spectra in the visible–near infrared and shortwave infrared (VNIR–SWIR) domain. In this regard, analysis of kimberlite rock spectra is carried out to understand how absorption features of its constituent minerals are preserved in the spectral profiles of two different types of kimberlites with distinct mineral assemblages; one serpentine-rich and the other carbonate-rich. It has been observed that the spectral signature of the rock is controlled by the diagnostic absorption features of dominant constituent minerals. However, wavelength and depth of the diagnostic absorption feature of the dominant constituent mineral are modified in the rock spectra due to nonlinear spectral mixing with spectral features of other constituent minerals of the rock. It is observed that the spectral profiles of the rocks are not influenced by the fabric and grain size variation, except for the variation in the albedo or background reflectance of the spectral profiles. Spectral features of the rock also remain stable with the changes in the spectral measurement parameters. Therefore, it is concluded that the rock spectra can be used as an end-member or reference for spatial mapping of the economic rock instead of its constituent minerals.

Keywords: End-member, fabric, grain size, kimberlite, spectral features.

Mapping of mineral deposits using ‘spectral’ features of minerals (significant to characterize the deposit) is well known. In this regard, airborne sensors such as Hymap,

ACKNOWLEDGEMENT. I thank the Indian National Science Academy (INSA), New Delhi for the award of INSA Honorary Scientist and Dr Rajesh Tandon for help in statistical analyses.

Received 12 September 2012; accepted 21 September 2012